Additional Records of Hemoparasites (Apicomplexa) and Helminth Parasites (Trematoda, Cestoda, Nematoda, Acanthocephala) from Oklahoma Amphibians (Anura) and Reptiles (Testudines: Ophidia)

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Abstract: Over the last few years, we have learned more about hematozoan (blood) parasites and helminth parasites of Oklahoma herpetofauna than we have gained in many years past. To that end, we continue to provide information on various intraerythrocytic hematozoans and helminths of amphibians and reptiles of the state. We document several new host and distributional records for these parasites.

Introduction

During the last decade, our research consortium has made an attempt to help fill a void in our knowledge of hematozoans (McAllister 2015; McAllister et al. 2018a) and helminths of Oklahoma's herpetofauna (McAllister and Bursey 2012; McAllister et al. 2014a, b, 2015, 2016, 2018a, b, and references therein). Here, we complement some of that information by reporting new host and distributional records for select parasites of amphibians and reptiles from southeastern Oklahoma.

Methods

Between May 2017 and July 2020, single adult specimens of Fowler's toad, *Anaxyrus fowleri* (Hinckley), southern leopard frog, *Rana sphenocephala* (Cope), snapping turtle, *Chelydra serpentina* (L.), eastern river cooter, *Pseudemys concinna concinna* (LeConte), southern black racer, *Coluber constrictor priapus* Dunn and Wood, broad-banded watersnake, *Nerodia fasciata confluens* (Blanchard), western ratsnake, *Pantherophis obsoletus* (Say), Dekay's brownsnake, *Storeria dekayi* (Holbrook), and two each eastern hog-nosed snakes, *Heterodon platirhinos* (Latreille), and northern cottonmouths, *Agkistrodon piscivorus* (Lacépède) were collected by hoop net, hand or snake tong from Choctaw County (n = 1) and from several sites in McCurtain County (n = 11), and examined for hematozoan and helminth parasites. Specimens were placed in cloth collection bags, placed in a refrigerator, and necropsied within 24 hr. They were measured for straight-line carapace length (CL) or snout-vent length (SVL), killed by an intraperitoneal injection of sodium pentobarbital (Nembutal®) following accepted guidelines (SIH 2004), and examined for hematozoan and helminth parasites. A bone saw was used to remove the plastron from turtles to expose the heart and a mid-ventral incision from mouth to cloaca was made to expose the same in amphibians and other reptiles. Blood was obtained from all specimens by making a small incision in their heart and taking a sample using ammonium heparinized (75 mm long) capillary tubes. Thin films were smeared onto microscopic slides, air-dried, fixed for 1 min in absolute methanol, stained for 20–30 min with Wright-Giemsa stain, and rinsed in phosphate buffer (pH = 7.0). Slides were scanned at 100× or 400× and when infected cells were found, photographs were taken and length and width (L × W) measurements were made on gamonts of an intraerythrocytic parasite (n = 20) using a calibrated ocular micrometer under a 1,000× oil immersion lens and are reported in micrometers as means ±1SD followed by the ranges. For intravascular trematodes in turtles, we followed methods of Snyder and Clopton (2005). All visceral organs, particularly those of the GI tract from all specimens, were examined for helminths by removing and splitting organs lengthwise, placing separate organs in a Petri dish with 0.9% saline, and their contents scanned at 20–30× using a stereomicroscope. The liver and other suspected infected tissues from two anurans and two snakes were also biopsied and specimens processed for examination by light microscopy following Presnell and Schreibman (1997). Trematodes and cestodes were fixed in nearly boiling tap water without coverslip pressure, stained with acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted in Canada balsam. Nematodes were fixed in hot tap water and studied as temporary mounts on a microscopic slide in a drop of glycerol. When acanthocephalans were found, they were rinsed of mucus and placed in dishes containing distilled water for 24 h in a refrigerator to evert their proboscides. Each specimen was placed on a glass slide, and a wet mount was prepared by adding a drop of tap water and a coverslip. All were examined at 100 to 400× with an Olympus BX-51 upright research microscope configured for Brightfield (BF) and Differential Interference-Contrast (DIC) microscopy. Digital images were taken of acanthocephalans using an Olympus 5-megapixel digital camera, and total length and greatest width of each worm were measured with ImageJ software (Schneider et al. 2012).

We follow the common and scientific names of North American herpetofauna of Crother (2017) except for adopting Yuan et al. (2016) in our usage of *Rana* rather than *Lithobates* for Oklahoma's ranid frogs. Host vouchers are deposited in the Arkansas State University Museum of Zoology (ASUMZ) Herpetological Collection, State University, Arkansas, or the Henderson State University Herpetological Collection (HSU), Arkadelphia, Arkansas. Actual vouchers or photovouchers of parasites are deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska.

Results and Discussion

Fourteen taxa of endoparasites, including three apicomplexans, four digeneans, two tapeworms, three nematodes, and two acanthocephalans were harbored by 14 hosts. An annotated list of the parasites found and the host data follows.

Apicomplexa: Adeleorina: Haemogregarinidae: Hepatozoidae

***Hepatozoon* sp. Miller, 1908** – Slender-elongate and slightly recurved gamonts of a *Hepatozoon* sp. (HWML 216370; Figs. 1A–B) was found in about 25% of the red blood cells (rbcs) of an adult *N. f. confluens* (675 mm SVL) collected on 10 June 2019 from an oxbow lake (Little River drainage), 3.2 km N of Idabel (33° 55' 56.93"N, 94° 43' 43.22"W). Gamonts were length × width, 18.5 × 7.1 (range 17–20 × 7–8) µm and caused a moderate hypertrophy to the host rbc by an increase in rbc length, but not width.

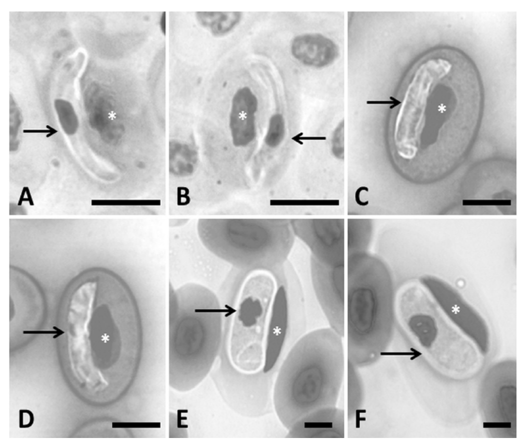
Lowichik and Yeager (1987) reported short and long "haemogregarine" gamonts in *N. f. confluens* from Louisiana, but unfortunately, did not provide photomicrographs. In addition, Telford et al. (2001) described *Hepatozoon pictiventris* in Florida watersnake, *Nerodia fasciata pictiventris* (Cope) from Florida. Gamonts (their figs. 25–26) were dissimilar to ours as they were elongate, but not slender and slightly recurved. This is the first time any haemogregarine has been reported from a *N. f. confluens* in Oklahoma along with the initial photomicrographs of the form observed in this host.

An adult *H. platirhinos* (650 mm SVL) collected on 15 October 2017 from Hochatown (34° 10' 17.0286"N, 94° 45' 05.7414"W) also harbored a few gamonts (Figs. 1C–D) of a *Hepatozoon* sp.; measurements were not taken and the other *H. platirhinos* was negative. Hilman and Strandtmann (1960) reported *H. serpentium* from three of four (75%) *H. platirhinos* from Texas. We report a *Hepatozoon* sp. from a *H. platirhinos* from Oklahoma for the first time.

A single adult *C. horridus* (1,030 mm SVL) collected on 10 May 2017 from the EOSC campus in Idabel (33° 55' 17.2632" N, 94° 46' 43.6548" W) were infected with gamonts (HWML 216371; Figs. 1E–F) of a *Hepatozoon* sp. McAllister (2015) previously reported *Hepatozoon* sp. from *C. horridus* collected from the same locale. However, a photomicrograph of the parasite was less than optimal and we here provide better photomicrographs from this host (Figs. 1E–F). Bean-shaped to elongate gamonts were prevalent that also resulted in the rbc nucleus becoming very elongate (Figs. 1E–F). Hematozoans have also been previously reported from *C. horridus* from New York (Fantham and Porter 1954) and *H. horridus* was described by (Telford et al. 2008) from timber rattlesnakes from Florida. The gamonts of *H. horridus* reported by the latter authors (their Figs. 5–6) do not resemble the current specimens.

Platyhelminthes: Digenea: Schistosomatoidea: Spirorchiidae

***Spirorchis scripta* Stunkard, 1923** – an adult *P. c. concinna* (320 mm CL) collected on 9 June 2019 from an oxbow lake (Little River drainage), 3.2 km N of Idabel (33° 55' 56.93"N, 94° 43' 43.22"W) had this digenean in its kidneys, liver and general body wash. *Spirorchis* spp. trematodes of turtles have a complex life cycle with turtles serving as



Figures 1A–F. Gamonts of *Hepatozoon* spp. from reptiles. (A–B) Gamonts from *Nerodia fasciata confluens*. (C–D) Gamonts from *Heterodon platirhinos*. (E–F) Gamonts from *Crotalus horridus*. Arrows indicate gamonts and asterisks (*) denote rbc nuclei; scale bars A–D = 10 µm; E–F = 5µm.

definitive hosts and snails as intermediate hosts. In the life cycle, for example, cercaria occurs in the planorbiid snails, the two-ridged ramshorn, *Helisoma anceps* (Holliman and Fisher 1968) and *Menetus dilatatus* (Goodchild and Martin 1969) and ancylid, *Ferrissia fragilis* (Turner and Corkum 1977). This blood fluke has previously been reported from *P. concinna* from Mississippi (Roberts et al. 2018) and Tennessee (Byrd 1939). It was originally described by Stunkard (1923) from Mississippi map turtle, *Graptemys pseudogeographica kohnii* (Baur) collected from Texas, and the red-eared slider, *Trachemys scripta elegans* (Wied-Neuwied) from North Carolina and has also been reported from the latter host from Oklahoma (Harwood 1931; Everhart 1975) as well as other turtles from Alabama, Georgia, Iowa, Nebraska, Ohio, Tennessee, Texas, Virginia, and Manitoba, Canada (Ernst and Ernst 1977; Timmers and Lewis, 1979; Roberts et al. 2016, 2019). However, this is the first time *S. scripta* has been reported from *P. c. concinna* in Oklahoma. Specimens are being retained for molecular analysis (VV Tkach, *pers. comm*.).

Plagiorchiida: Plagiorchiidae

***Styphlophora magna* Byrd and Denton, 1938** – Three *S. magna* was found in the gallbladder of an *A. piscivorus* (427 mm SVL) collected on 23 June 2019 from the same Hochatown site above. An additional *A. piscivorus* (510 mm SVL) collected from the same site on 5 September 2020 had a single *S. magna* in its gallbladder. Byrd and Denton (1938) described *S. magna* from the gallbladder of northern watersnake, *Nerodia sipedon sipedon* (L.) from Georgia and Mississippi. A single specimen from the gall bladder of Florida kingsnake, *Lampropeltis floridana* (Blanchard) from Florida was reported by Byrd et al. (1940). More recently, Fontenot and Font (2011) reported *S. magna* from Mississippi green watersnake *N. cyclopion* (Duméril, Bibron and Duméril), southern watersnake, *N. fasciata* (L.), northern diamond-backed watersnake, *N. rhombifer rhombifer* (Hallowell) and *A. piscivorus* from Louisiana. We document the second report of *S. magna* from *A. piscivorus* but more importantly, the first documentation of this digenean from west of the Mississippi River in Oklahoma. Specimens are being utilized in a molecular study (VV Tkach, *pers. comm.*).

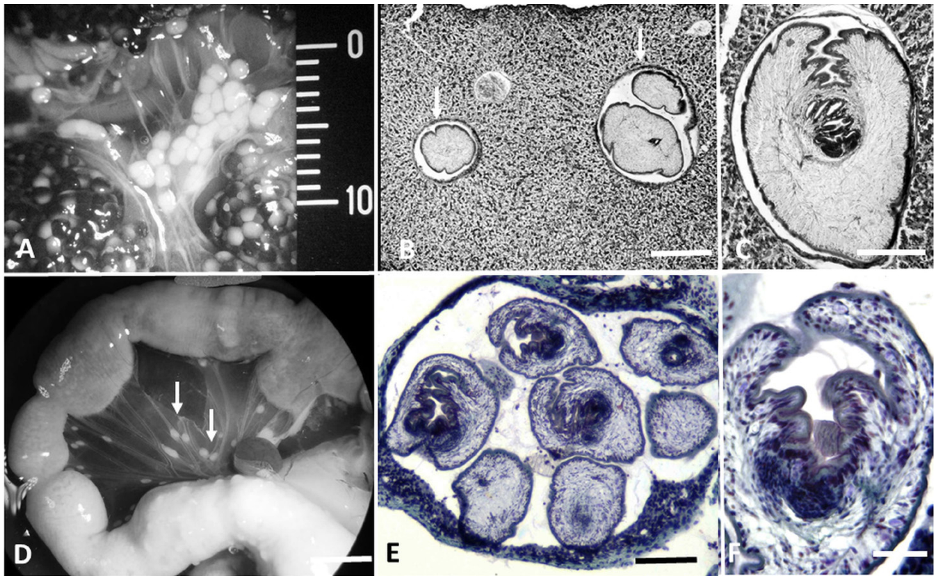
Ochetosomatidae

***Dasymetra conferta* Nicoll, 1911 –** Four *D. conferta* were found in the esophagus of an adult (1,020 mm SVL) *P. obsoletus* collected on 15 July 2020 from the same Hochatown site above. This trematode has been previously reported from Oklahoma in *N. r. rhombifer*, plain-bellied watersnake, *Nerodia erythrogaster* (Forster) and *H. platirhinos* (McAllister and Bursey, 2012; McAllister et al. 2016, 2018a). The parasite's range also includes hosts from Alabama, Missouri, and Texas (see Ernst and Ernst 2006). The western ratsnake is a new host record for *D. conferta*. Specimens are being utilized in a molecular study (TJ Fayton, *pers. comm.*).

***Renifer* sp. –** Six specimens of a *Renifer* sp. were found in the oral cavity of the same *N. f. confluens* above. Two species, *R. aniarum* (Leidy, 1891) and *R. magnum = magnus* (Byrd and Denton, 1938) have been reported from *N. f. confluens* (Ernst and Ernst 2006; Fontenot and Font 1996). The former taxon was reported from this host from Louisiana (Rabalais 1969; Brooks 1979; Fontenot and Font 1996) and from a *N. r. rhombifer* from Oklahoma (McAllister and Bursey 2012). However, this is the first time this host has been reported with a *Renifer* sp. from Oklahoma. The genus is badly in need of revision including molecular analyses, so we wait until a more definitive diagnosis can be made to identify our taxon here. These specimens have been retained for DNA studies (TJ Fayton, *pers. comm.*)

Cestoda: Cyclophyllidea: Mesocestoididae

***Mesocestoides* sp.** – two anuran and a snake species were found to possess tetrathyridia of *Mesocestoides* sp. One anuran host, an adult *R. sphenocephala* (80 mm SVL) collected on 25 August 2019 from Hochatown (34° 10' 17.0286"N, 94° 45' 05.7414"W) had tetrathyridia (HWML 216369) in its liver (Figs. 2A–C). *Mesocestoides* has previously been reported from *R. sphenocephala* from Arkansas by McAllister et al. (2014b). The other anuran

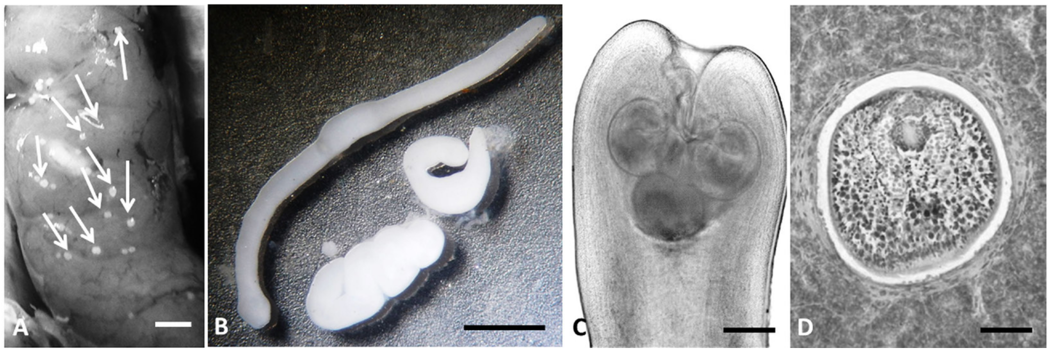


Figures 2A–F. *Mesocestoides* sp. tetrathyridia from anurans; note uniform size and shape with fully invaginated normal scoleces with suckers and normal organized body (C, F). (A) Multiple tetrathyridia in peritoneal cavity of *Rana sphenocephala*. (B) Individual tetrathyridium or dual tetrathyridia (arrows) in liver parenchyma of *R. sphenocephala*. (C) Single tetrathyridium from liver of *R. sphenocephala*. (D) Tetrathyridia (arrows) in omental tissue of *A. fowleri*. (E) Multiple tetrathyridia (n = 7) in single host-derived capsule of *A. fowleri*. (F) Single tetrathyridium from *A. fowleri*. Scale bars (A) each mark = 1 mm; (B) 1 mm; (C) 250 µm; (D) 5 mm; (E) 250 µm; (F) 250 µm.

host, an adult *A. fowleri* (60 mm SVL) collected from the same locality on 20 September 2016 was also found to possess tetrathyridia of *Mesocestoides* sp. (HWML 216368) in its liver (Figs. 2D–F) and mesenteries. This cestode was reported previously from *A. fowleri* in Arkansas and Michigan (McAllister et al. 2014b; Muzzall and Andrus 2014). It is being reported here for the first time in these particular host species from Oklahoma. These specimens are being processed further for molecular analysis of the genus *Mesocestoides* in amphibians (VV Tkach, *pers. comm.*). The tetrathyridia from both anuran hosts were uniform in size and shape, probably indicating similar age or time from initial infection (see McAllister et al. 2018b).

The same adult *H. platirhinos* collected from the same Hochatown locality above in October 2017 harbored *Mesocestoides* sp. tetrathyridia (Figs. 3A–C). We report *Mesocestoides* sp. tetrathyridia from this host in Oklahoma for the first time. The tetrathyridia from this host were of two distinct size and shape categories, with one group being small and rounded, and the other being larger and elongated, possibly indicating different ages or two distinct infection incidents (see McAllister et al. 2018b).

Given the frequent fact that some isolates of *Mesocestoides* tetrathyridia from various parts of the world are known to proliferate asexually, it is important to note that none of our specimens in this study showed any sign of asexual activity or abnormal development. On the contrary, all specimens exhibited highly organized musculature, parenchyma, and excretory systems, with no sign of supernumerary scoleces as described for *Mesocestoides vogae* Etges 1991, or distended excretory ducts and hyperplastic tegumentary invaginations associated with malignant transformation in aberrant proliferative *Mesocestoides lineatus* (Goeze, 1782) and other species (Conn et al. 2010, 2011; Conn 2016). Thus, our findings are consistent with all other field collections of *Mesocestoides* we have reported over many years from diverse hosts in North America



Figures 3A–D. *Mesocestoides* sp. tetrathyridia and *Spirometra* sp. from *Heterodon platirhinos*; note variations in size and shape of *Mesocestoides* sp. (A) Multiple tetrathyridia in situ (arrows) in liver. (B) Three individual tetrathyridia from peritoneal cavity or teased from liver capsules. (C) Anterior end of tetrathyridium; note fully invaginated normal scolex with four suckers and normal organized body. (D) *Spirometra* sp. (encapsulated plerocercoid) in liver tissue. Scale bars (A) 1 mm; (B) 2mm; (C) 100 µm; (D) 250 µm.

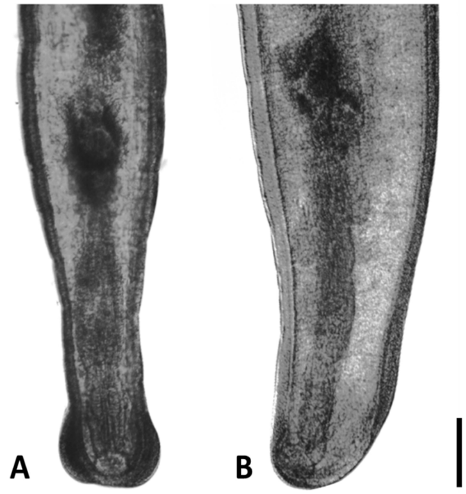
(McAllister et al. 1989, 1991a, b, c, 1992, 1995, 2004, 2005, 2017, 2018b). The only exception in three decades of our studies on the genus was a single moribund western coachwhip, *Coluber flagellum testaceus* Say, *in* James from Texas that harbored unidentified aberrant acephalic metacestodes co-occurring with normal non-proliferating *Mesocestoides* tetrathyridia (Conn and McAllister 1990); the aberrant forms in that unusual case were possibly not *Mesocestoides*, but, unfortunately, were unidentifiable due to lack of scoleces.

This enigmatic cestode, for whom no complete life cycle is known, has been previously reported from other Oklahoma herpetofauna, including Sequoyah slimy salamander, *Plethodon sequoyah* Highton, Hurter's spadefoot, *Scaphiopus hurterii* Strecker, plains spadefoot, *Spea bombifrons* (Cope), American bullfrog, *Rana catesbeianus* (Shaw), little brown skink, *Scincella lateralis* (Say, *in* James), and eastern gartersnake, *Thamnophis sirtalis sirtalis* (L.) (see McAllister and Bursey 2004; McAllister et al. 2005, 2017, 2018a, b). It is of interest to note that although the complete life cycle remains an enigma, it appears that from the numerous specimens (both frogs and snakes) collected from the identical Hochatown site that harbor tetrathyridia of this cestode, some unknown member or members that serve in the initial part of the life cycle occurs at this site. Further research is certainly warranted to help investigate this query.

Diphyllobothriidea: Diphyllobothriidae

***Spirometra* sp.** – Another adult *H. platirhinos* (650 mm SVL) collected in April 2020 from the same Hochatown locality also harbored this cestode in its liver (Fig. 3D) and body cavity.

The life cycle requires three hosts, members of the genus reproduces in wild and feral canines and felines and other mammals, but can also cause subcutaneous, visceral, ocular, and cerebral pathologies and zoonosis (sparganosis, a foodborne and waterborne disease) in humans, the majority of cases reported in China and Korea (Hwang et al. 2020). In some species within this genus, the vertebrate second intermediate host (and paratenic host) may be a frog, snake, bird, or mammal, and the first intermediate host is a copepod (Kuchta et al. 2015, 2020). At least four species are currently recognized (Kuchta and Scholz 2017); however, our metacestodes cannot be identified to species without molecular analyses. The forms in the Americas (both North and South America) are split into two groups that are currently recognized as the *S. decipiens* complex (Kuchta et al. 2020). Nevertheless, we document a new host and the first report of the genus in snakes from Oklahoma. Specimens from *H. platirhinos* are being processed further for molecular analysis of the genus *Spirometra* in reptiles (VV Tkach, *pers. comm*.).



Figures 4A–B. ***Neoechinorhynchus*** **spp. from** ***Pseudemys concinna concinna*****. (A) Posterior ends of non-gravid female** ***Neoechinorhynchus pseudemydis*** **and (B)** ***Neoechinorhynchus emyditoides*****. Scale bar = 100 µm.**

Ascaridida: Cosmocercidae

Cosmocercoides dukae **(Holl, 1928) Travassos, 1931** – Twenty-four female and seven male specimens (HWML 111603) were taken from the lower intestine of a *S. dekayi* (125 mm SVL) collected on 15 May 2020 from Sweethome, N of Broken Bow (34° 03' 22.1796"N, 94° 46' 27.8832" W). *Cosmocercoides variabilis* (Harwood, 1930) Travassos, 1931 was reported previously from *S. dekayi* from Oklahoma by McAllister et al. (2015). However, the current specimens are clearly *C. dukae* as they possess 12 pairs of plectanes, not the 16 or more possessed by *C. variabilis*. The former was reported by Harwood (1932) from *S. dekayi* from Texas. The life cycle involves terrestrial gastropods as intermediate hosts and amphibians and reptiles as definitive hosts (Anderson 2000). This is the first report of *C. dukae* in a *S. dekayi* from Oklahoma.

Nematoda: Strongylidea: Diaphanocephaloidea: Diaphanocephalidae

Kalicephalus inermis coronellae (Ortlepp, 1923) Lichtenfels, 1980 – A single *C. c. priapus* collected on 15 May 2019 from Broken Bow (34° 01' 7.4316" N, 94° 45' 25.3512" W) had one female *K. i. coronellae* (HWML 112120) in its esophagus. Ernst and Ernst (2006) previously listed *C. constrictor* as a host of this nematode but its subspecific identity was not provided; 11 subspecies of *C. constrictor* have been recognized (see Crother 2017). McAllister et al. (2018a) reported this nematode from Oklahoma in prairie kingsnake, *Lampropeltis calligaster* (Harlan). This hookworm has been reported from at least 25 snake (both colubrid and viperid) species from Colorado, Florida, Georgia, Louisiana, Massachusetts, New Mexico, North Carolina, and Texas, and Québec, Canada, and Guerrero, Michoacán, and Vera Cruz, México (Schad 1962; Baker 1987; Ernst and Ernst 2006).

Ascaridida: Anisakidae

Contracaecum **sp. (larvae)** – two larval *Contracaecum* sp. (HWML 112116) were found deeply embedded in the intestinal mucosa of an adult (260 mm CL) *C. serpentina* collected on 1 October 2019 from a private lake in Ft. Towson, Choctaw County (34° 08' 32.5752" N, 95° 20' 38.4756" W). Crustaceans (primarily copepods and amphipods) serve as first intermediate hosts, freshwater and marine fishes are second intermediate/paratenic hosts, and fish-eating birds and mammals are definitive hosts (Anderson 2000). This is the first time the larvae have been reported from *C. serpentina*. *Contracaecum* larvae similar to our specimens have been reported from Florida softshell, *Apalone ferox* (Schneider) in Florida (Foster et al. 1998).

Acanthocephala: Eoacanthocephala: Neoechinorhynchida: Neoechinorhynchidae

Neoechinorhynchus emyditoides **Fisher, 1960 –** the same *P. c. concinna* reported above harboring *S. scripta* was found to be infected with a non-gravid female of *N. emyditoides* (HWML 112102) in its lower intestine. The length and width (L × W) of *N. emyditoides* was 12.9 × 0.44 mm, respectively.

Neoechinorhynchus pseudemydis **Cable and Hopp, 1954 –** a non-gravid female of *N. pseudemydis* (HWML 112103) was also found in the same host above. The L × W of *N.*

pseudemydis was 16.9 × 0.56 mm, respectively. Both acanthocephalans were identified based on posterior end morphology (Figs. 4A–B) (Barger and Nickol 2004).

Previously, *P. concinna* has been reported with *N. emydis* (Leidy, 1851) Van Cleave, 1916 from Illinois, *N. emyditoides* from Louisiana, and *N. chrysemydis* Cable and Hopp, 1954 from Alabama (as *P. c. hieroglyphica* × *concinna*), Louisiana, North Carolina, and an unknown locality (Van Cleave 1919; Johnson 1968; Acholonu 1969; Barger 2004). Additionally, McAllister et al. (2015) reported non-gravid acanthocephalans of either *N. pseudemydis* and/or *N. emyditoides* from *P. c. concinna* from Oklahoma, supporting the current findings. Here, we report a new host record for *N. pseudemydis* in *P. c. concinna*. *Neoechinorhynchus pseudemydis* has been reported from at least six species of turtles, including *C. serpentina*, painted turtle, *Chrysemys picta* (Schneider), red-eared slider, *T. s. elegans,* Blanding's turtle, *Emydoidea blandingii* (Holbrook), *Kinosternon* sp., and gopher tortoise, *Gopherus polyphemus* (Daudin) (Ernst and Ernst 1977; Barger 2004). These results strengthen the need to survey *P. c. concinna* for acanthocephalans, with future efforts dedicated to molecular identification.

In summary, we report some new host and geographic records for these parasites. There remains a need to continue surveying the diverse herpetofauna of the state, especially species found in the western part of Oklahoma that has been rarely examined. Additional new host and geographic distribution records are to be expected with extensive surveys, including the possibility of discovering novel taxa.

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